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Award Number: DAMD17-03-1-0729

TITLE: Healing of Stress Fracture in an Animal Model

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REPORT DATE: September 2004

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
               Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
                        Distribution Unlimited

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REPORT DOCUMENTATION PAGE

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1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE September 2004	3. REPORT TYPE AND DATES COVERED Annual (1 Sep 2003 – 31 Aug 2004)

4. TITLE AND SUBTITLE
Healing of Stress Fracture in an Animal Model

5. FUNDING NUMBERS
DAMD17-03-1-0729

6. AUTHOR(S)

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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 Words)

This project aims to develop effective ways to prevent or treat stress fractures. We have successfully established a reproducible stress fracture model in the rodent. We have investigated the role of an exercise program in the prevention of stress fractures using the ulna axial compression loading model. Loading was applied on right ulnas using a 2-Hz haversine waveform with a peak force of 17 N for 360 cycles/day, three days per week for five consecutive weeks. After adaptation to mechanical loading was determined by assessing both the material and structural properties of each ulna, ulnas (loaded vs. nonloaded) were loaded at a constant peak load of 25 N using a 5 Hz haversine waveform until fatigue failure. Loading improved both the material and structural properties of the ulna, especially minimum second moment of area increasing about 2-fold. Loaded ulnas failed in fatigue after 1.3 million cycles, compared to the 16,850 cycles required for non-loaded ulnas, suggesting that the mechanical loading program increased the fatigue resistance by 80-fold. In addition, animal experimental parts regarding the individual and combined roles of anti-inflammatory agent (cyclooxygenase-2 inhibitor) and low-intensity pulsed ultrasound in the treatment of stress fractures were done.

14. SUBJECT TERMS
stress fracture, exercise, Loading, cyclooxygenase-2 inhibitor, therapeutic ultrasound

15. NUMBER OF PAGES
8

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Unlimited

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Introduction

This project aims to develop effective ways to prevent or treat stress fractures. The project includes 2 studies:

Study One:
  (1) A preliminary loading (exercise) program on the incidence of stress fractures in the rodent ulna and;
  (2) Loading (exercise) bouts on the incidence of stress fractures in the rodent ulna.

Study Two:
  (1) COX-2 inhibition on the timecourse of healing of rodent ulna stress fractures;
  (2) LIPUS on the timecourse of repair of rodent ulna stress fractures and;
 (3) Combined use of a COX-2 inhibitor and LIPUS on stress fracture repair in the rodent ulna.

Body

Study one:

1. A preliminary loading (exercise) program on the incidence of stress fracture
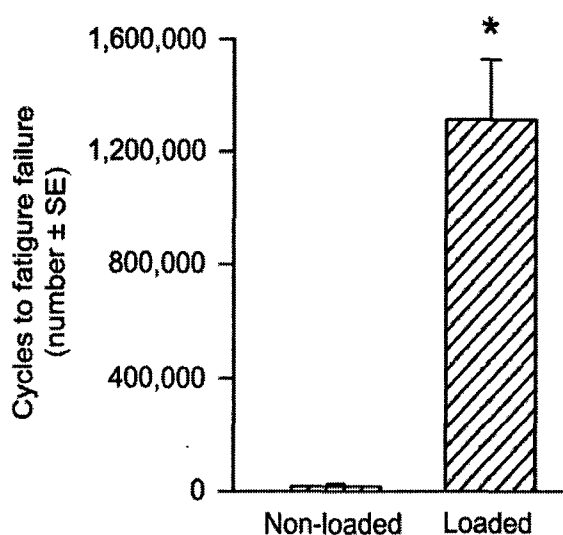
We have investigated the role of an exercise program in the prevention of stress fractures using the ulna axial compression loading model. Loading was applied on right ulnas of rats using a 2-Hz haversine waveform with a peak force of 17 N for 360 cycles/day, three days per week for five consecutive weeks. Adaptation to mechanical loading was determined by assessing both the material and structural properties of each ulna. Loading improved both the material and structural properties of the ulna, especially minimum second moment of area (Imin) increasing about 2-fold (**Fig. 1**).

First, ulnas (loaded vs. nonloaded) were loaded at a constant peak load of 25 N using a 5 Hz haversine waveform until fatigue failure. The load of 25 N produces 3614 ± 409 $\mu\epsilon$ on the midshaft of loaded ulnas, compared to the 5361 ± 618 $\mu\epsilon$ on nonloaded ulnas ($p <$ 0.05, paired t-test). The loaded ulnas failed in fatigue after 1.3 million cycles, compared to the 16,850 cycles required for non-loaded ulnas (**Fig. 2**), suggesting that the mechanical loading program increased the fatigue resistance by 80-fold. These data have been submitted as an abstract to 51st annual meeting of Orthopedic Research Society at 2005 (See Appendices).

Second, ulnas (loaded vs. nonloaded) were loaded at two different peak loads using a 5 Hz haversine waveform until fatigue, which produce the same strain on both loaded and nonloaded ulnas. These data are currently being analyzed.

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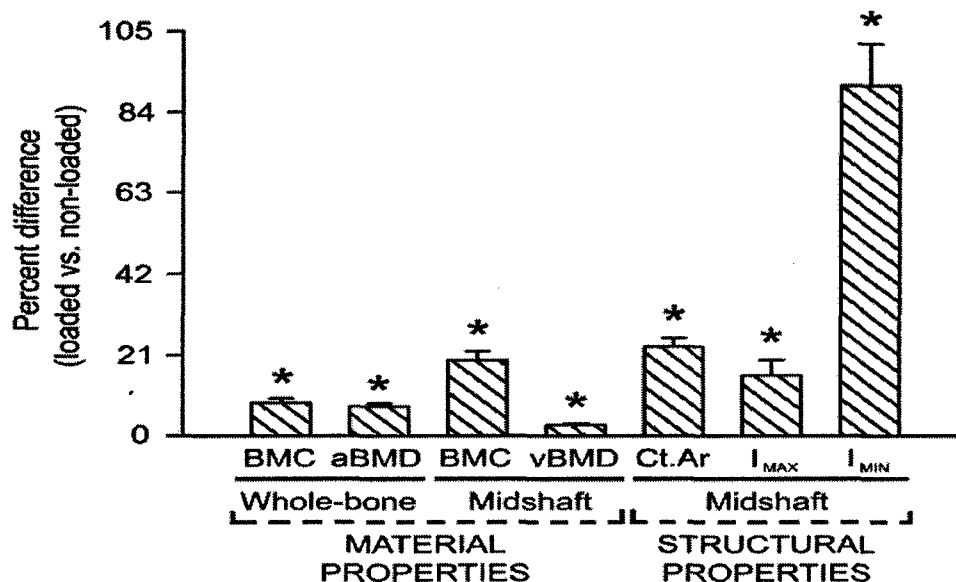
Fig. 1. Effect of exercise on the material properties (BMC: bone mineral content and BMD: bone mineral density) and structural properties (Ct.Ar: cortical area; Imax and Imin) of the ulna. *Indicates significant difference from 0% (no difference between loaded and non-loaded ulnas) ($p < 0.05$; single sample t-test with population mean of 0%). Error bars represent ± SE.

Fig. 2. Effect of exercise-induced bone changes on ulna fatigue resistance. *Indicates significant difference from non-loaded ulna ($p < 0.01$, paired t-test). Error bars represent ±SE.

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2. Loading (exercise) bouts on the incidence of stress fractures in the rodent ulna.
This experiment will be started soon.

Study two:

Animal experimental parts regarding the individual and combined roles of anti-inflammatory agent (cyclooxygenase-2 inhibitor) and low-intensity pulsed ultrasound in the treatment of stress fractures were done. The bone specimens are currently being processed and will be analyzed in 2 months.

We modified the grouping as shown in Table 1. Tami et al (1) has reported that fracture line is remodeled and disappears after 42 days using a similar animal model. So we adjusted the time course to 2, 4 and 8 weeks in our study, instead of 12 weeks after stress fracture. Celecoxib (3-4 mg/kg/day), a specific COX-2 inhibitor like NS-398, has been shown to delay fracture repair (2, 3). Celecoxib, whose commercial name is Celebrex, has been widely used in clinics. We think it will be more clinically relevant to use Celecoxib, compared with NS-398 that cannot be used clinically. Therefore, we chose Celecoxib in our study instead of NS-398.

Table 1. Study 2: Combined effects of LIPUS and a COX-2 inhibitor on stress fracture repair

Groups*	COX-2 inhibitor treated		Vehicle (placebo) treated	
	Active-ultrasound treated	Inactive-ultrasound treated	Active-ultrasound treated	Inactive-ultrasound treated
Baseline	Right ulnas	Left ulnas	Right ulnas	Left ulnas
2 weeks	Right ulnas	Left ulnas	Right ulnas	Left ulnas
4 weeks	Right ulnas	Left ulnas	Right ulnas	Left ulnas
8 weeks	Right ulnas	Left ulnas	Right ulnas	Left ulnas

* Time following stress fracture induction.

Overall, our project has been going smoothly. We have not encountered any specific difficulties.

Key Research Accomplishments

1. Complete the experiment to study a preliminary loading (exercise) program on the incidence of stress fracture. Part of the data was submitted to 51st annual meeting of Orthopedic Research Society at 2005. The other data are currently being analyzed;

2. Complete the animal experimental parts regarding the individual and combined roles of anti-inflammatory agent (cyclooxygenase-2 inhibitor) and low-intensity pulsed ultrasound in the treatment of stress fractures. The specimens are currently being processed.

Reportable outcomes

1. An abstract, titled by "BONE ADAPTATION TO A MECHANICAL LOADING PROGRAM SIGNIFICANTLY INCREASES SKELETAL FATIGUE RESISTANCE", was submitted to 51st annual meeting of Orthopedic Research Society at 2005;
2. Rodent ulnar axial compression loading model was established;
3. Two college students supported by this award took part in the project.

Conclusions

By inducing adaptation of its material and structural properties, a mechanical loading program can increase the fatigue resistance of the rat ulna by 80-fold. This large increase occurred despite relatively low gains in aBMD and BMC because load-induced new bone is localized to biomechanically relevant sites.

Stress fractures are frequently seen among military recruits causing a valuable loss of training time and a substantial financial burden (4). Our findings indicate that a preliminary exercise program can significantly increase skeletal fatigue resistance. It suggests that a preliminary exercise program before the intensive training can significantly decrease the incidence of stress fracture in infantry. Our data will have significant clinical implications with regard to the prevention of stress fracture.

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Appendices
See next page.

BONE ADAPTATION TO A MECHANICAL LOADING PROGRAM SIGNIFICANTLY INCREASES SKELETAL FATIGUE RESISTANCE

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INTRODUCTION: A stress fracture represents the failure of a bone to withstand repetitive bouts of mechanical loading. Despite the clinical significance of stress fractures in terms of loss of training and competition time, there are few effective preventative strategies. One hypothesized method is to use an exercise program to modify the material and structural properties of an at risk bone. Bone is an inherently mechanosensitive tissue that adapts its properties in response to the prevailing mechanical environment. By inducing adaptation through a mechanical loading program, a bone's resistance to fatigue may be enhanced resulting in a subsequent reduction in stress fracture risk. However, the ability of a loading program to reduce stress fracture risk has not been established. The aim of this study was to investigate whether the material and structural adaptation of bone to a mechanical loading program can improve skeletal fatigue resistance in an animal model.

METHODS: Adult female Sprague-Dawley rats (250-300g) were used, and all procedures were approved by an Institutional Animal Care and Use Committee. Site-specific mechanical loading was achieved using the ulna axial compression loading model, with the animals under isoflurane anesthesia (Abbott Laboratories, North Chicago, IL). This mode of loading accentuates the mediolateral curvature of the ulna and translates most (approximately 90%) of the axial load into a bending moment to create a strain distribution similar to that resulting from normal limb usage during locomotion. Loading was applied using a 2-Hz haversine waveform for 360 cycles/day, three days per week for five consecutive weeks. The peak load magnitude was 17 N, which elicits a compressive strain of approximately 3600 $\mu\varepsilon$ on the medial surface of the ulna midshaft. Left ulnas served as an internal control and were not loaded. Normal cage activity was allowed between loading sessions. Animals were killed five weeks following the final loading session.

Adaptation to mechanical loading was determined by assessing both the material and structural properties of each ulna. Material properties were assessed using dual energy X-ray absorptiometry (DXA) and peripheral quantitative tomography (pQCT). DXA was performed to collect whole ulna bone mineral content (BMC; mg) and areal bone mineral density (aBMD; g.cm^{-2}) data, whereas pQCT was performed to determine ulna midshaft BMC (mg.cm^{-1}) and volumetric BMD (vBMD; mg.cm^{-3}). Structural properties of each ulna midshaft were derived from micro-computed tomography (μCT) slices. Slice images were imported into Scion Image wherein ulna midshaft cortical area (Ct.Ar; mm^2), and the maximum (I_{MAX}; mm^4) and minimum (I_{MIN}; mm^4) second moments of area were determined. In addition, the section diameter (Se.Dm; mm) of each ulna was determined as the largest diameter of the bone in the plane parallel to the I_{MIN} axis.

To permit the calculation of microstrain ($\mu\varepsilon$), right (loaded) and left (non-loaded) ulnas from four animals chosen at random were used for a load-strain calibration experiment. A single element strain gauge (EA-06-015DJ-120; Measurements Group, Inc., Raleigh, NC) was bonded at the midpoint of each ulna, and the ulnas were axially loaded at four different peak loads. The peak-to-peak gauge voltage was converted to strain, and a graph was constructed plotting $\mu\varepsilon$/N versus c/I_{MIN}, where c is 2/3 of the Se.Dm. The slope of this graph (0.018 $\mu\varepsilon$/N/mm^{-3}) was used to predict strain during fatigue testing in experimental ulnas from their I_{MIN} and c using the derived equation:

$$\mu\varepsilon = \frac{N \times c}{0.018 \times I_{MIN}}$$

Remaining ulna pairs were loaded at a constant peak load of 25 N until fatigue failure. For testing, ulnas were fixed with 0.5 N of preload between two opposing cup-shaped platens on an electromagnetic actuator (Bose® ElectroForce® 3200 series; EnduraTEC, Minnetonka,

MN) and axially loaded in load control. Loading was performed continuously in a room temperature saline bath using a 5 Hz haversine waveform. Upon fatigue failure the number of completed cycles was recorded for each bone.

RESULTS: The mechanical loading program had predictable and consistent effects on the material and structural properties of the ulna, with loaded ulnas having significantly greater values for all measurements compared to the contralateral non-loaded ulnas (Fig. 1). The largest difference was observed for I_{MIN}, which was near two-fold greater in exercised ulnas. As a result of the adaptation in loaded ulnas, they experienced only 3614 ± 409 $\mu\varepsilon$ when loaded at 25 N during fatigue testing, which compared to the 5361 ± 618 $\mu\varepsilon$ experienced by nonloaded ulnas ($p < 0.05$, paired t-test).
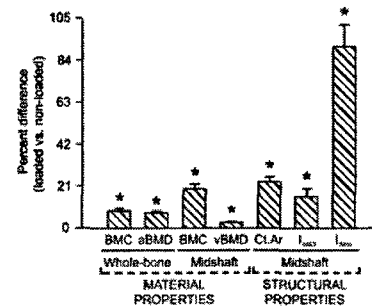
Fig. 1. Effect of exercise on the material and structural properties of the ulna. *Indicates significant difference from 0% (no difference between loaded and non-loaded ulnas) ($p < 0.05$; single sample t-test with population mean of 0%). Error bars represent ± 1 SE.

The material and structural adaptation of the ulna to mechanical loading significantly increased ulna fatigue resistance. Loaded ulnas failed in fatigue after 1.3 million cycles, compared to the 16,850 cycles required for non-loaded ulnas (Fig. 2). This represents a 80-fold increase in ulna fatigue resistance resulting from the mechanical loading-induced bone adaptation.

Fig. 2. Effect of exercise-induced bone changes on ulna fatigue resistance. *Indicates significant difference from non-loaded ulna ($p < 0.01$, paired t-test). Error bars represent ± 1 SE.
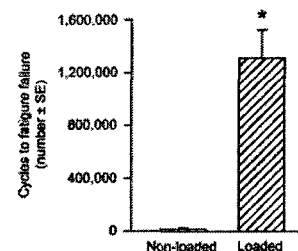
DISCUSSION: By inducing adaptation of its material and structural properties, a mechanical loading program increased the fatigue resistance of the rat ulna by 80-fold. This large increase occurred despite relatively low gains in aBMD and BMC because load-induced new bone was localized to biomechanically relevant sites. Axial compression of the rat ulna accentuates its mediolateral curvature to generate strain and subsequent adaptation in the mediolateral plane. As this plane corresponds with the plane of I_{MIN}, adaptation to the mechanical loading program was predominantly observed as an increase in I_{MIN}. By fatigue loading the ulnas in the same direction as they were adapted, the two-fold increase in I_{MIN} resulted in a substantial increase fatigue resistance.